would indicate their usefulness in aqueous or nonaqueous lubricants. They also seem to have some use as viscosity control agents in such systems. The lubricity of the alkylphenyl ether or ester derivatives, even at relatively dilute concentrations at a metal-metal interface, is remarkable. They will provide useful lubrication at pressures from 2–2½ times as great as that provided by comparable soap solutions widely used in the wire drawing and metal extruding industries. In addition, their nonionic character would probably eliminate the buildup of sludge in such systems, particularly in the drawing of copper tubing and wire.

Derivatives prepared by substituting alkylphenyl group for the methyl group at the hydrophobic end of the polyoxyethylene alcohol show promise as textile lubricants to prevent the balling of fabrics in presses. It is also expected that they would impart a better hand to the fabric due to their many ester groups which seem to be useful in this respect. The low foaming characteristics of the methyl ethers could lead to their use in heavy duty power washing. Inasmuch as there are functional groupings present after the products have been formed, their use as chemical intermediates is also suggested. One interesting family of derivatives is that prepared by splitting the triglyceride formed with a primary or secondary amine. The diethanolamides of these materials formed in this manner exhibit unique properties in that, while still being diethanolamides, the resulting products have more greatly reduced effects on the viscosity of aqueous solutions than do the corresponding derivatives of saturated fatty acids or unsaturated fatty acids. Their use as chemical intermediates has, however, of this date been just barely investigated.

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Chromatographic Analysis of Sucrose Esters of Long Chain Fatty Acids

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Abstract

The procedure of the quantitative analysis of the components of sucrose ester products is described. By thin-layer chromatography, sucrose ester products have been separated into their components: mono-,di-,tri-, and higher esters. The individual components have been extracted from the plate and determined colorimetrically by using anthrone reagent. Standard substances of monoesters, diesters and triesters, which have been synthesized from gas-chromatographically pure methyl esters of fatty acids, have been isolated by column chromatography and identified by elemental analysis, colorimetric determination and N.M.R. determination.

Introduction

RECENTLY, nonionic surfactants have been developed and a number of studies of their physical properties have been reported. In most of these surfactants, hydrophilic properties are due to polyoxyethylene chains, which have a range of molecular weight distributions. It is almost impossible to obtain surface-chemically pure samples from such surfactants.

In 1956, sucrose esters of long chain fatty acids, such as sucrose laurate, myristate, palmitate, and stearate were prepared by Osipow et al. (1). These esters have a hydrophilic group of definite molecular structure, viz., a sucrose residue. This will permit a supply of pure samples for further studies of the physicochemical properties of nonionic surfactants.

As in usual organic reactions, it is assumed that the preparation of pure monoesters or diesters is not obtained. Therefore, mixtures of sucrose, sucrose monoester, sucrose diester, and others must be formed. To obtain pure samples, fatty acid methyl esters must be purified and sucrose ester products separated into their components. Consequently, before syntheses of sucrose esters, fatty acid methyl esters were purfiied by distillation and gas-chomatographically pure methyl esters were obtained. By using these methyl esters, sucrose esters were prepared and pure mono- or di-esters were isolated by silica gel column chromatography.

In the early stages of study on the synthesis of sucrose esters, it was believed that sucrose, which was the raw material for these esters, was the lowest cost hydrophilic group available for use in the preparation of nonionic surfactants (2). To manufacture sucrose esters at low cost it is necessary to examine the conditions of manufacture and to control the product. For these purposes, an easy and accurate method to analyze sucrose ester products was required.

Only one method for the analysis of sucrose ester has been reported (1). This method, however, requires large samples and must be carried out with the assumption that sucrose ester products contain sucrose, mono- and di-esters, and no tri- and higher esters.

The quantitative determination of the components of sucrose ester products was accomplished by a minor modification of TLC described by Vioque et al. (3).

Experimental

Samples. Sucrose esters were synthesized by Osipow's method from gas-chromatographically pure methyl esters of fatty acids.

Anthrone Reagent. Anthrone was dissolved in con-

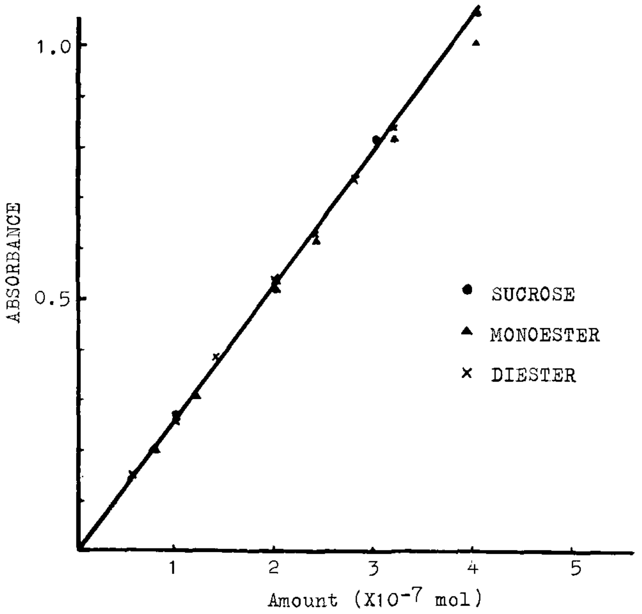

Fig. 1. Calibration curve for sucrose, sucrose monopalmitate and sucrose dipalmitate.

centrated sulfuric acid to give a 0.2% (w/v) solution.

Silica Gel. Mallinckrodt A.R., 100 mesh.

Solvent. Solvents used were reagent grade.

Color Reaction. The quantitative determination of sucrose using anthrone reagent was suggested by Dreywood (4) and developed by Morse (5). This method was used with some improvement in this work. The procedure follows: Two ml of an aqueous solution, which contained 0.03–0.15 mg sucrose, was placed in a test tube. Then 5 ml of anthrone reagent was added slowly from a pipet, cooled with water, and shaken vigorously. During this process temp of the mixtures must not exceed 70C for 30 min. Below this temp color formation is slow, while above this temp the color is unstable. The color obtained was measured at 625 $m\mu$. A blank was prepared by substituting distilled water for the sample solution.

Investigation as to whether the anthrone reagent was applicable for determination of sucrose ester products under similar conditions led to satisfactory results.

Quantitative Analysis of Esters by Employing TLC

TLC was used to separate the sucrose ester products into their components (6).

Chromatography. Plates were prepared according to the method of Davidek et al. (7) using dry silica gel as adsorbent. The size of the plates was 20 × 15 cm. Layer thickness was ca. 1 mm.

The individual components in the products could be separated on the plate by developing with 25% ethanol in benzene or isopropyl alcohol in benzene. The sample was dissolved in alcohol to ca. 1% solution (if the solution was cloudy a small amount of benzene and/or water was added).

The sample solution was applied as a spot ca. 2–3 cm from one end of the start line as reference, and as a few spots (0.03–0.05 ml) from the other end as sample.

The plate was slanted in a large Petri dish (30 cm diam) and developed by the ascending technique until the solvent reached the top of the plate. Usually, about 1 hr was sufficient.

Detection and Extraction. After developing, the solvent was allowed to evaporate. Anthrone reagent was sprayed on the reference side of the plate, whereupon the spots on the reference side were colored green on a yellow background by heating. Each zone on the sample side, corresponding to the colored spots of the reference side, was scraped from the plate with a spatula into a funnel. Each scraped silica gel was extracted 5 times with ca. 2 ml of ethanol into a test tube.

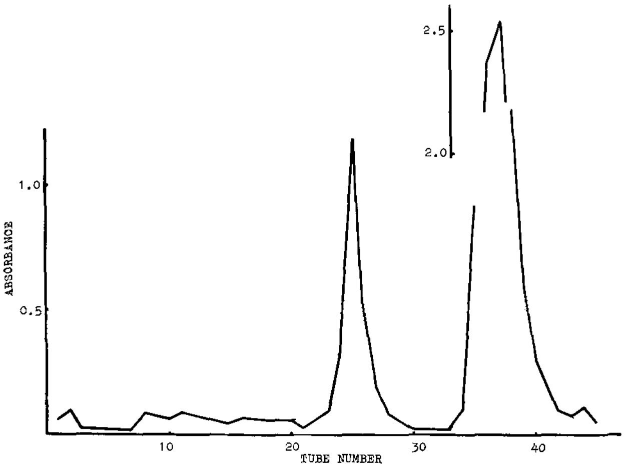

Fig. 2. Elution curves of monopalmitate product colored with anthrone reagent.

Determination. Extracts were evaporated to dryness in a water bath, 2 ml of distilled water was added, and the solution treated as described above.

Isolation of Pure Esters

Column Chromatography. Based on data of TLC, column chromatography was attempted. The column was 1 cm diam and 20 cm long, with a porous plate at the bottom.

Eight g of silica gel was weighted in a beaker and mixed with 15 ml of 29.5% iso-amyl alcohol in benzene. The slurry was transferred to the column. When the solvent level reached top of the silica gel column, 0.5 g of a sample dissolved in the solvent was put into the top of the column. The stepwise elution method was used. The eluent mixtures used were:

1) 20 ml of 29.5% iso-amyl alcohol in benzene.
2) 20 ml of 25.0% iso-propyl alcohol in benzene.
3) 60 ml of 25.0% ethanol in benzene.

Fifty fractions, each containing 700 mg of effluent, were collected, using an automatic fraction collector. The amount of material in each fraction was determined colorimetrically after evaporating the solvent.

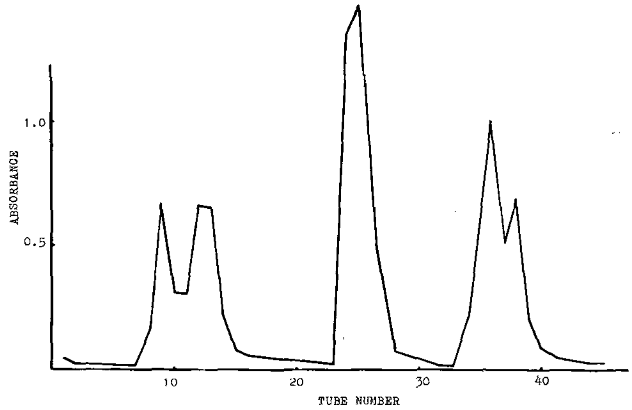

Fig. 3. Elution curves of dipalmitate product colored with anthrone reagent.

TABLE I
Results of Elemental Analysis

	Calculated		Observed	
	C	H	C	H
Monopalmitate	57.91	9.03	58.19	9.13
Dipalmitate	64.51	10.09	64.27	10.03
Tripalmitate	68.14	10.68	67.99	10.66
Monolaurate	54.95	8.45	55.12	8.64
Monomyristate	56.51	8.75	56.38	9.07

TABLE II
N.M.R. of Sucrose Palmitate
Ratio of the intensity of proton

	Calculated		Observed	
	Sucrose residue	Fatty acid chain	Sucrose residue	Fatty acid chain
Monopalmitate	14	31	14	32.6
Dipalmitate	14	62	14	60.8

Results and Discussion

Calibration Curve. Solutions containing known amounts of sucrose, sucrose monopalminate, and sucrose dilpalmitate were subjected to the above procedure. As shown in Figure 1, the intensities of color per mole are all equal for sucrose, sucrose monoester, and diester. Moreover, since this color reaction is attributed to mono-saccharide unit the intensity of color per mole of sucrose is twofold that of glucose or fructose.

Isolation of Pure Esters. Examples of the elution curves of sucrose mono- and dipalmitate products are given in Figures 2 and 3. They plot absorbance against tube number. The first peak from the right side of the curve corresponds to monoester, and the second to diester. The third peak in Figure 3, which consisted of two cusps, has not been investigated. As the fractions contain triester, so it may be triester and higher esters, or two isomers of triester.

Individual fractions, i.e., sucrose mono- and diester were developed again on TLC and identified as pure mono- and diester, respectively. The monoester thus obtained was readily soluble in water and was very foamy, while the diester was only dispersed in water and almost insoluble.

Further identification of the materials was accomplished by three methods:

1) Elemental analysis of C and H (analytical values were given in Table I).
2) Colorimetric determination due to sucrose residue (cf. Fig. 1).
3) N.M.R. determination (integral intensity for sucrose residue and for fatty acid chain were compared and results shown in Table II).

TABLE III
Recovery Test of TLC Method
One % alcoholic solutions of mono- and di-palmitate products employed for determination respectively

Sample	Volume taken	Method	Result absorbance	Recovery %
Mono-palmitate product	0.04 ml	a) colored directly	1.627	95.3
		b) colored after separation by TLC	S 0.3534 M 0.9830 D 0.2146	
			Total 1.5510	
Di-palmitate product	0.04 ml	a) colored directly	1.224	105.6
		b) colored after separation by TLC	S 0.1628 M 0.4288 D 0.4406 T 0.2606	
			Total 1.2928	

Results are the mean values from 5 experiments.
S: sucrose, M: sucrose monopalmitate, D: sucrose dipalmitate, T: sucrose tripalmitate.

TABLE IV
Reproducibility expressed in absorbance

		1	2	3	4	5	avg	SD
Mono-palmitate product	S	0.353	0.349	0.319	0.403	0.343	0.3534	0.0123
	M	0.924	1.015	1.022	0.962	0.992	0.9830	0.0162
	D	0.252	0.211	0.181	0.191	0.238	0.2146	0.0121
	Total	1.529	1.575	1.522	1.556	1.573	1.5510	0.0098
Di-palmitate product	S	0.130	0.166	0.169	0.160	0.189	0.1628	0.0085
	M	0.449	0.449	0.377	0.440	0.429	0.4288	0.0122
	D	0.441	0.429	0.445	0.448	0.440	0.4406	0.0029
	T	0.292	0.280	0.243	0.270	0.218	0.2606	0.0119
	Total	1.312	1.324	1.234	1.318	1.276	1.2928	0.0152

S: sucrose, M: sucrose monopalmitate, D: sucrose dipalmitate, T: sucrose tripalmitate.

Quantitative Determination

Test of Method. To test the recovery of sucrose esters in the extraction procedure which forms a part of the method, the yield of color per wt of sucrose ester products was compared with analytical values obtained by the method employing TLC. One per cent alcoholic solution of sucrose mono- and dipalmitate products were prepared respectively. Exactly 0.01 ml of this solution was injected into a test tube from a micropipet, the solution was evaporated, 2 ml of distilled water was added, and the solution was colored by anthrone reagent method as described above. The color thus obtained was due to sucrose and sucrose residues which were contained in sucrose monoester, diester, and others. For easy comparison of results, fourfold values, that is the absorbance of the color obtained with 0.04 ml of solution, are shown in Table IIIa. On the other hand, 0.04 ml of the same solution was put on the thin layer from the micropipet, each component of the sucrose ester products was separated by TLC, and analyzed by the process above and color reaction. Results obtained are given in Table IIIb. From Table III, it is clear that recovery is ca. 95–105%.

For the examination of the reproducibility of the method, sucrose mono- and dipalmitate products were analyzed five times respectively. Results obtained with these two samples are shown in Table IV. It is noted that reproducibilities for each component in the series of the table are less than that for the total values. This means that the scrapping procedure of each zone of components from the plate is the accuracy-determining process in this method. Thus the accuracy is ca. 95%.

Skilled technique was required for this procedure because of the incomplete method for detecting each zone of components. More accurate results can be obtained if a more complete detection method is available.

Analysis of Sucrose Esters. Other sucrose esters, e.g., sucrose laurate, myristate, and stearate can be analyzed similarly.

This method is also applicable, with some modification, to the analysis of raffinose esters of fatty acids, e.g., raffinose oleate products were determined separately by using 29.5% ethanol in benzene on the plate.

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